COMMONWEALTH OF AUSTRALIA

PATENTS ACT 1952-69

Form 10

COMPLETE SPECIFICATION

61,470/73 (ORIGINAL)

FOR OFFICE USE :

Application Number : 61470/73
Lodged : 17·10·73

Class
09·65-1
87·16

Int. Class: C07C 103/52 // A61K 25/00

Complete Specification Lodged : 17·10·73
~~Accepted~~ : Lapsed Section 47c (b)
Published : 17·4·75

Priority : Switzerland 24·11·72 17184/72

Related Art :

Name of Applicant : F.HOFFMANN-LA ROCHE & CO. AKTIENGESELLSCHAFT

Address of Applicant : 124-184 Grenzacherstrasse, Basle, Switzerland

Actual Inventors: DIETER GILLESSEN, JOSEF RUDINGER and ROLF STUDER

Address for Service : Spruson & Ferguson, Patent Attorneys, Esso House, 127 Kent Street, Sydney, New South Wales, 2000 Australia.

Complete Specification for the invention entitled :

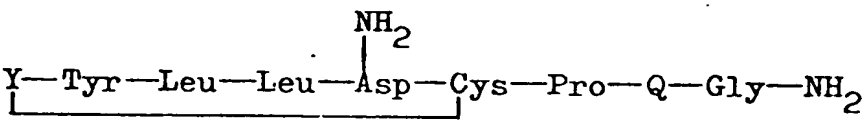
"PEPTIDES"

The following statement is a full description of this invention, including the best method of performing it known to me/us :

Journal
20/11/74 K

The present invention is concerned with polypeptides and a process for the manufacture thereof.

The polypeptides provided by the present invention are compounds of the general formula



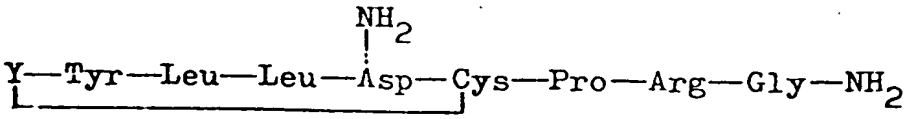
(I)

, wherein Q represents the residue of arginine or lysine and

Y represents the residue of cysteine, of β-mercaptopropionic acid (Mpr) or Gly—Cys— and wherein all amino acids with an asymmetric centre have the L-configuration,

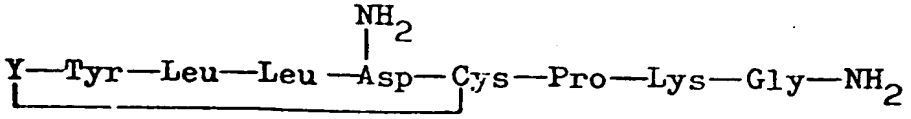
and pharmaceutically acceptable, non-toxic acid addition salts thereof.

The compounds of formula I, which can also be represented by the formulae



(Ia)

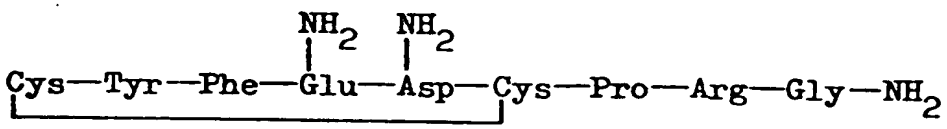
and



(Ib)

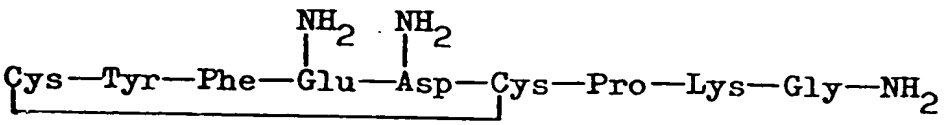
, wherein Y has the significance given earlier,

are related to analogues and their derivatives of the naturally occurring neurohypophysial hormones; for example, the arginine- or lysine-vasopressin of the formulae



Cys—Tyr—Phe—Glu(NH$_2$)—Asp(NH$_2$)—Cys—Pro—Arg—Gly—NH$_2$ (IIa)

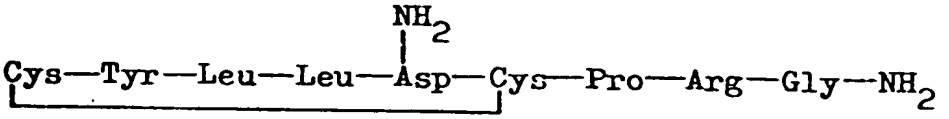
and



Cys—Tyr—Phe—Glu(NH$_2$)—Asp(NH$_2$)—Cys—Pro—Lys—Gly—NH$_2$ (IIb)

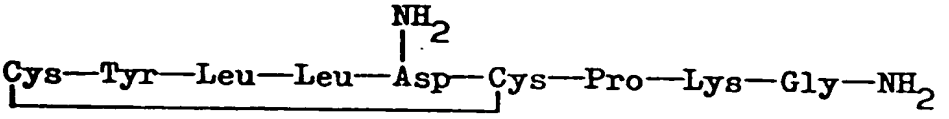
The compounds provided by the present invention differ from the naturally occurring vasopressins by the replacement of the amino acid phenylalanine by leucine, the amino acid glutamine by leucine and, optionally, the amino acid cysteine by β-mercaptopropionic acid or the dipeptide glycylcysteine.

The compounds falling within formula I are the following:



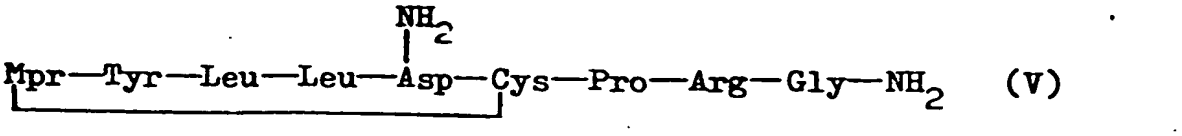
Cys—Tyr—Leu—Leu—Asp(NH$_2$)—Cys—Pro—Arg—Gly—NH$_2$ (III)

= [Leu^3, Leu^4]-Argininevasopressin



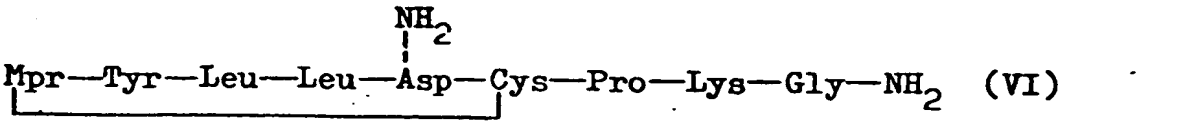
Cys—Tyr—Leu—Leu—Asp(NH$_2$)—Cys—Pro—Lys—Gly—NH$_2$ (IV)

= [Leu^3, Leu^4]-Lysinevasopressin



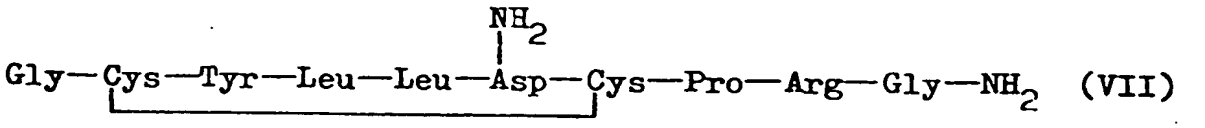
(V)

= Deamino[1]-[Leu[3], Leu[4]]-argininevasopressin



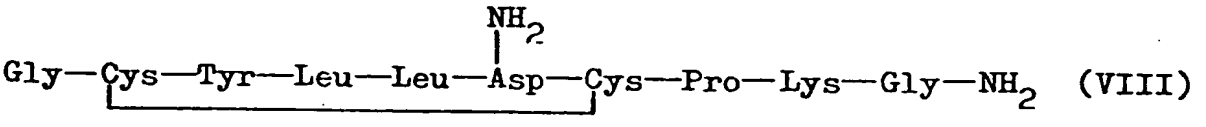
(VI)

= Deamino[1]-[Leu[3], Leu[4]]-lysinevasopressin



(VII)

= Gly-[Leu[3], Leu[4]]-argininevasopressin



(VIII)

= Gly-[Leu[3], Leu[4]]-lysinevasopressin

The abbreviations used in the present specification for the individual amino acids and their protecting groups are those hitherto customarily used in peptide chemistry and generally known to the person skilled in the art [Literature: Schröder, E. and Lübke, K.,: The Peptides, Academic Press, New York & London, Vol. I (1965) and Vol. II (1966) and IUPAC-IUB Rules]. No further definition of such abbreviations is therefore given in this specification.

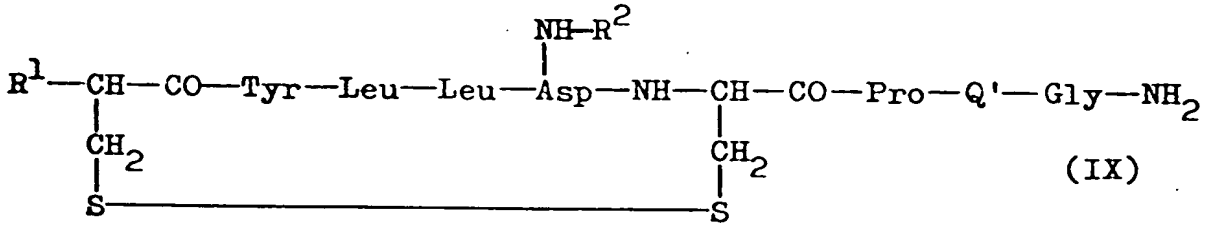
In general, β-mercaptopropionic acid, which is derived from cysteine, is likewise considered in the present specification to be an "amino acid" so that, for example, β-mercaptopropionyl-tyrosine is said to be a dipeptide etc.

Unless expressly stated otherwise, the amino acids with an asymmetric centre always have the L-configuration.

Examples of pharmaceutically acceptable, non-toxic acid addition salts are salts formed with inorganic acids such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulphuric acid and perchloric acid or with organic acids such as acetic acid, oxalic acid, maleic acid, malic acid, tartaric acid or citric acid.

According to the process provided by the present invention, the polypeptides aforesaid (i.e. the compounds of formula I and their pharmaceutically acceptable, non-toxic acid addition salts) are manufactured by

a) cleaving off the protecting group(s) from a peptide of the general formula



(IX)

, wherein R^1 represents a hydrogen atom or a grouping of the formula $R^{11}-NH-$,

R^{11} represents a hydrogen atom, an amino protecting group or an optionally protected glycyl residue,

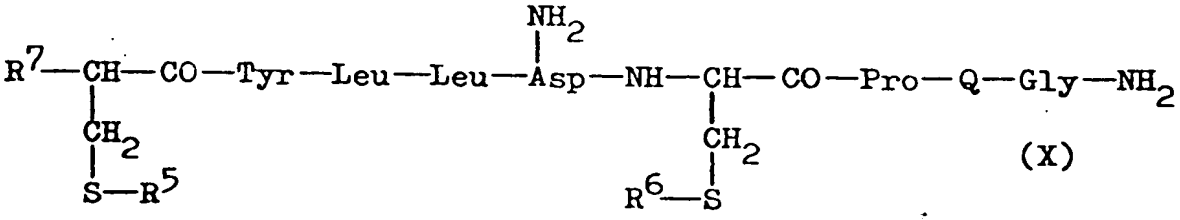
R^2 represents a hydrogen atom or an amide protecting group,

Q' represents a grouping of the formula
$-NH-CH[-(CH_2)_3-NH-C(=NH)-NHR^3]-CO-$ or
$-NH-CH[-(CH_2)_4-NH-R^4]-CO-$,
R^3 represents a hydrogen atom or a group protecting the guanidine residue and
R^4 represents a hydrogen atom or a group protecting the ε-amino group of lysine, provided that at least one of R^1, R^2 and R^3 or R^4 represents or contains a protecting group, and wherein all amino acids with an asymmetric centre have the L-configuration,

and, if desired, converting the free peptide obtained into a pharmaceutically acceptable, non-toxic acid addition salt by reaction with an organic or inorganic acid,

or

b) oxidising a peptide of the general formula

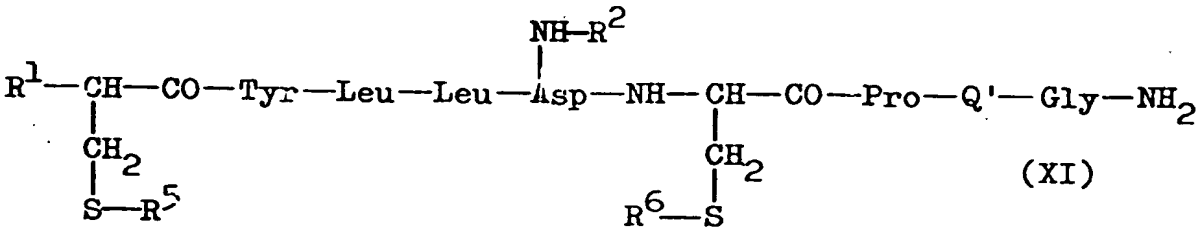


(X)

, wherein Q has the significance given earlier,
R^5 and R^6 each represent a hydrogen atom or a sulphydryl protecting group and
R^7 represents a hydrogen atom or the grouping H_2N- or Gly-NH- and wherein all amino acids with an asymmetric centre have the L-configuration,

with simultaneous or prior cleavage of protecting groups which may be present and, if desired, converting the product obtained into a pharmaceutically acceptable, non-toxic acid addition salt by reaction with an organic or inorganic acid, or

c) oxidising a peptide of the general formula



(XI)

, wherein R^1 represents a hydrogen atom or a grouping of the formula $R^{11}-NH-$,

R^{11} represents a hydrogen atom, an amino protecting group or an optionally protected glycyl residue,

R^2 represents a hydrogen atom or an amide protecting group,

Q' represents a grouping of the formula $-NH-CH[-(CH_2)_3-NH-C(=NH)-NHR^3]-CO-$ or $-NH-CH[-(CH_2)_4-NH-R^4]-CO-$,

R^3 represents a hydrogen atom or a group protecting the guanidine residue,

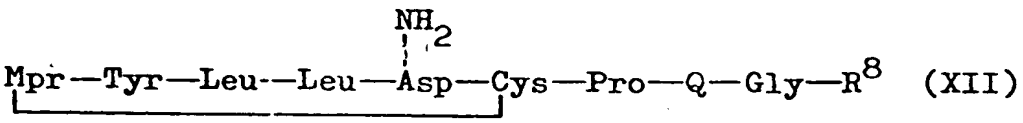
R^4 represents a hydrogen atom or a group protecting the ε-amino group of lysine and

R^5 and R^6 each represent a hydrogen atom or a sulphydryl protecting group, provided that at least one of R^1, R^2 and R^3 or R^4 represents or contains a protecting group, and wherein all amino acids with an asymmetric centre have the L-configuration,

with simultaneous cleavage of the protecting group(s) and, if desired, converting the product obtained into a pharmaceutically acceptable, non-toxic acid addition salt by reaction with an organic or inorganic acid,

or

d) amidating a compound of the general formula



(XII)

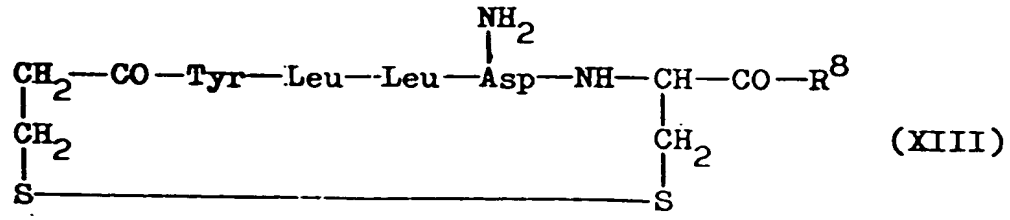
, wherein Q has the significance given earlier,

R^8 represents a hydroxy group or a moiety activating the carboxyl group and

Mpr represents the residue of β--mercaptopropionic acid,

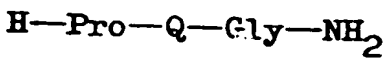
or

e) reacting a hexapeptide of the general formula



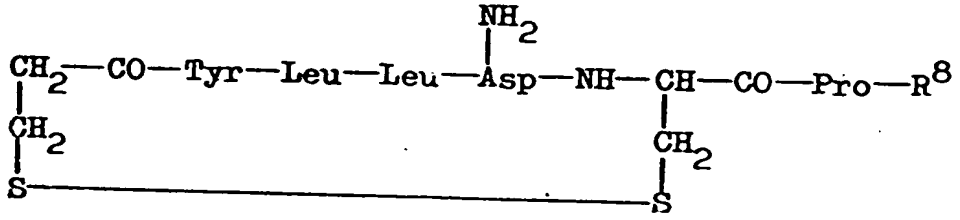
(XIII)

with a tripeptide of the general formula



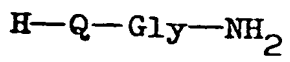
(XIV)

or reacting a heptapeptide of the general formula



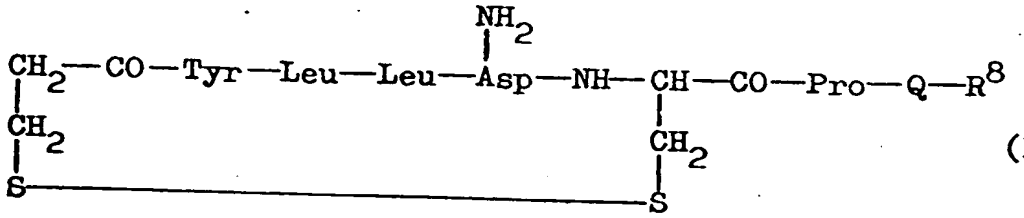
(XV)

with a dipeptide of the general formula



(XVI)

or reacting an octapeptide of the general formula



(XVII)

with glycinamide and, if desired, converting the resulting nonapeptide into a pharmaceutically acceptable, non-toxic acid addition salt, in formulae XIV and XVI Q having the significance given earlier and in formulae XIII, XV and XVII R^8 representing a hydroxy group or a moiety activating the carboxyl group and all amino acids with an asymmetric centre having the L-configuration.

The oxidation of a peptide of formula X or XI can be carried out in a manner known per se (see, for example, Schröder-Lübke, Vol. I, page 275 et seq). It is preferably carried out in an

aqueous or aqueous/organic solution by the introduction of air or oxygen or by means of hydrogen peroxide, iodine, 1,2--diiodoethane or potassium ferricyanide. Sulphydryl protecting groups, which may be present, can be removed simultaneously with or prior to the oxidation. The oxidation of a peptide of formula X in which R^5 and R^6 both represent a hydrogen atom or a trityl, benzhydryl, acetamidomethyl, benzylthiomethyl or isobutyloxymethyl group can be carried out, for example, with dirhodane [$(SCN)_2$] and the oxidation of a peptide of formula X in which R^5 and R^6 both represent a hydrogen atom or a trityl or acetamidomethyl group can be carried out, for example, with iodine.

The cleavage of protecting groups from a peptide of formula IX or XI can also be carried out in a generally known manner and under the conditions applicable to the individual groups.

The amidation of a compound of formula XII, especially one in which Q represents the residue of arginine, can be carried out in a manner known per se, preferably by carefully reacting an activated ester with aqueous ammonia at room temperature.

The protecting groups referred to in this specification can be any of the protecting groups known in peptide chemistry.

Examples of amino protecting groups are those of the acyl type (e.g. formyl, benzoyl, phthalyl, trifluoroacetyl, p-tosyl, aryl- and alkyl-phosphoryl, phenyl- and benzyl-

sulphonyl, tritylsulphenyl, o-nitrophenylsulphenyl, γ-chlorobutyryl and o-nitrophenoxyacetyl), of the alkyl type (e.g. trityl, benzyl and alkylidene) or of the urethane type (e.g. carbobenzoxy, p-bromo-, p-chloro- or p-methoxy-carbobenzoxy, tolyloxy-, allyloxy-, cyclopentyloxy, cyclohexyloxy-, t-butyloxy- or 1,1-dimethylpropyloxy-, 2-(p-biphenylyl)-2--propyloxy-carbonyl and benzylthiocarbonyl). In addition, amino groups can be protected by protonation. Examples of amide protecting groups are xanthenyl, 2,4-dimethoxybenzyl, 2,4,6-trimethoxybenzyl and 4,4'-dimethoxybenzhydryl.

Special protecting groups for the arginine residue include, for example, p-tosyl, carbobenzoxy, p-nitrocarbobenzoxy, t-butoxy-, adamantyloxy- or isobornyloxycarbonyl. The arginine residue can also be protected by protonation or nitration.

Examples of sulphydryl protecting groups are alkylthio and arylthio groups such as ethylthio, t-butylthio and phenylthio, alkyl- and substituted-alkyl groups such as t-butyl, 2-diethoxycarbonyl-ethyl, benzyl, trityl, p-methoxybenzyl, p-nitrobenzyl, 4-picolyl, benzylthiomethyl, acetamidomethyl and isobutyloxymethyl, acyl groups such as carbobenzoxy, benzoyl, acetyl, p-methoxy-benzyloxycarbonyl and ethylaminocarbonyl or tetrahydropyran-2-yl.

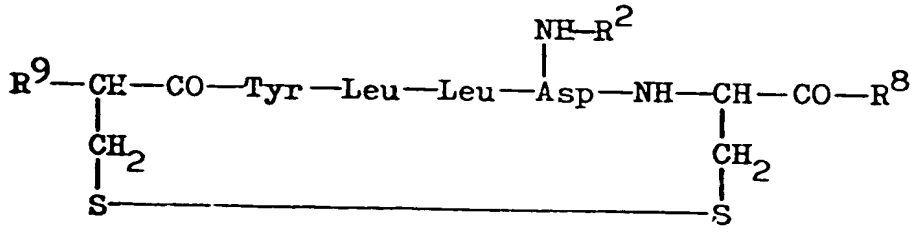
The starting materials of formulae IX, X, XI, XII, XIII, XV and XVII are novel and it will be appreciated that they also form part of this invention.

The starting materials can be prepared in a manner known per se using the usual protecting groups, especially those mentioned earlier.

Examples of carboxyl protecting groups are O- and S-esters (e.g. the methyl, ethyl, t-butyl, benzyl, cyanomethyl, phthalimidomethyl, 4-picolyl, 2-p-tosylethyl, phenyl, p-nitrophenyl, thiophenyl and p-nitrobenzyl esters), amides or hydrazides (e.g. the trityl, phenyl, carbobenzoxy and t-butoxycarbonyl hydrazides). In addition, the carboxyl group can be protected by salt formation.

Examples of activated carboxyl groups are esters such as the cyanomethyl, p-cyanophenyl, p-nitrophenyl, 2,4,5--trichlorphenyl, thiophenyl, p-nitrothiophenyl, 1-benztriazolyl, phthalimidyl, 1-succinimidyl, 1-piperidyl, 8-quinolyl, 5-chloro--8-quinolyl, 2-pyridyl and 2-thiopyridyl esters or azides.

A peptide starting material of formula X or XI can be prepared, for example, by the successive chain-lengthening of a dipeptide with an amino acid unit or from two or more basic units. A peptide of formula XI can be converted into a peptide of formula IX by oxidation in a manner known per se. A peptide of formula IX can, however, also be prepared, for example, by reacting a compound of the general formula



(XVIII)

, wherein R^2 and R^8 have the significance given earlier and

R^9 represents a hydrogen atom, a protected amino group or a protected glycylamino residue,

with a tripeptide of the general formula

$$H—Pro—Q'—Gly—NH_2 \qquad (XIX)$$

wherein Q' has the significance given earlier.

A compound of the formula XII can be prepared, for example, by reacting a compound of formula XIII in which R^8 represents an activated ester group with a tripeptide of the general formula

$$H—Pro—Q—Gly—OH \qquad (XX)$$

, wherein Q has the significance given earlier, and, if desired, converting the reaction product into an activated ester in a manner known per se.

A compound of formula XVIII in which R^9 represents a hydrogen atom can, however, also be readily converted into a hexapeptide of formula XIII by removal of the amide protecting group. The heptapeptides of formula XV and the octapeptides of formula XVII can be prepared, for example, by reacting a hexapeptide of formula XIII with a compound of the formula Pro—R^8 or Pro—Q—R^8 in which R^8 and Q have the significance given earlier.

The manufacture of the compounds of formula I according to the 6+3, 7+2 or 8+1 principle, as well as by amidation, is especially preferred for the argininevasopressin analogues.

The polypeptides provided by the present invention have hormonal activity qualitatively similar to that of the neurohypophysial hormones. The strong natriuretic activity is especially prominent. They are superior, not only with regard to the strength of action but also with regard to the duration of action, to natural argininevasotocin [[Ile^3]--argininevasopressin] and to the [Leu^4]-oxytocin prepared by V. J. Hruby et al [J. Biol. Chem. 244, 3890 (1969)] which is a neurohypophysial hormone analogue which had hitherto the strongest known natriuretic activity. The hypertensive activity of the present polypeptides is less than that of argininevasotocin, the natriuretic activity of the present polypeptides being selectively increased with respect to the hypertensive activity.

[Leu^3, Leu^4]-argininevasopressin has a TRF_{Na} [Tubular Rejection Fraction of Na according to the method of Cort et al, A. J. of Physiol. 215 (1968) 921] in the cat of 6.9% at 20 µg/kg and a half-life of the duration of action of 40 minutes. Deamino^1-[Leu^3, Leu^4]-argininevasopressin has a TRF_{Na} of 4.3% at 20 µg and a half-life of the duration of action of 45 minutes. Gly-[Leu^3, Leu^4]-argininevasopressin has a TRF_{Na} of 5.2% at 50 µg and a half-life of the duration of action of 45 minutes.

On the basis of the aforementioned biological activities, the present polypeptides are suitable for the treatment of oedemas of various types and of general disorders of electrolyte exchange, especially those of sodium retention.

The dosage of the present polypeptides should be regulated according to the individual requirements and can vary between 100 µg and 10 mg per single dose which may be administered one or more times per day.

The present polypeptides can be administered in the form of free bases or as pharmaceutically acceptable, non-toxic salts with organic or inorganic acids or with polymers containing acid groups (e.g. carboxymethylcellulose or tannic acid). The polypeptides may be administered alone or in the form of pharmaceutical preparations suitable, for example, for oral, parenteral, enteral or intranasal application. For the production of pharmaceutical preparations, the polypeptides can be compounded with inorganic or organic adjuvants which are inert and physiologically acceptable.

Examples of such adjuvants are:

for tablets: lactose, starch, talc and stearic acid;

for injection solutions: water, alcohols, glycerin and vegetable oils;

for suppositories: natural and hydrogenated oils and waxes;

for intranasal spray solutions: water, glycerin and other liquid substances which are tolerated by the mucous membrane.

The preparations can also contain, for example, suitable preservatives, stabilisers and wetting agents as well as sweetening, colouring and flavouring materials.

It will accordingly be appreciated that the invention includes within its scope a pharmaceutical preparation containing a polypeptide as hereinbefore defined in association with a compatible pharmaceutical carrier.

The following Examples illustrate the process provided by this invention:

Example 1

(a) Z-L-Leucyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl--N^G-tosyl-L-arginyl-glycinamide.

18.0 g of Z-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl--N^G-tosyl-L-arginyl-glycinamide [prepared according to R. L. Huguenin and R. A. Boissonnas, Helv. 49, 695 (1966)] were dissolved in 100 ml of glacial acetic acid and treated with 100 ml of a 5 N hydrogen bromide/glacial acetic acid solution. The mixture was stirred for 45 minutes at room temperature and subsequently added dropwise to 1 litre of ether. The precipitated hydrobromide of the pentapeptide was washed with ether, dried over potassium hydroxide and phosphorus pentoxide and dissolved in 100 ml of methanol. The solution was passed through a column of Dowex 2 (OH^- form), the eluate concentrated under reduced pressure and the residue dissolved in 100 ml of dimethylformamide. The solution was treated at 0°C with 8.5 g of Z-L-Leu-OPhNC$_2$, the mixture stored for 3 days at room temperature and the protected hexapeptide precipitated by the addition of 1 litre of ethyl acetate, washed with ether and ethyl acetate and dried. Yield: 16.3 g; melting point 183°-185°C; $[\alpha]_D^{25} = -41.6°$ (c = 0.5 in dimethylformamide).

(b) Z-L-Leucyl-L-leucyl-L-asparaginyl-S-benzyl-L-cysteinyl--L-prolyl-N^G-tosyl-L-arginyl-glycinamide.

The Z-protecting group was cleaved off from 5.0 g of Z-L-leucyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-N^G--tosyl-L-arginyl-glycinamide in the manner described in part (a) and the free amine obtained was reacted with 1.85 g of Z-L-Leu-$OPhNO_2$ in 50 ml of dimethylformamide. The mixture was stored for 3 days at room temperature, the protected heptapeptide precipitated by the addition of ethyl acetate, filtered off, washed with ethyl acetate and ether and dried. Yield: 4.5 g; melting point 188°-189°C; $[\alpha]_D^{25} = -41.2°$ (c = 1 in dimethylformamide).

(c) Tos-S-Benzyl-L-cysteinyl-O-benzyl-L-tyrosine methyl ester.

A solution of 21.9 g of Tos-S-benzyl-L-cysteine, 19.3 g of O-benzyl-L-tyrosine methyl ester hydrochloride and 6.73 ml of N-methylmorpholine in 200 ml of dimethylformamide was treated at 0°C with 1.30 g of dicyclohexylcarbodiimide, stirred for 30 minutes at 0°C and for a further 4 hours at room temperature and stored for 15 hours at 4°C. The precipitate was filtered off and the filtrate concentrated under reduced pressure. The residue was dissolved in ethyl acetate and the solution washed three times each with 1 N hydrochloric acid, saturated sodium chloride solution, saturated sodium bicarbonate solution and saturated sodium chloride solution, dried and concentrated under reduced pressure. The residue was crystallised from ethanol/hexane. Yield: 25.7 g; melting point 111°-112°C; $[\alpha]_D^{25} = +2.9°$ (c = 1 in methanol).

(d) Tos-S-Benzyl-L-cysteinyl-O-benzyl-L-tyrosine hydrazide.

18 g of Tos-S-benzyl-L-cysteinyl-O-benzyl-L-tyrosine methyl ester were dissolved with warming in 150 ml of ethanol. The solution was treated with 7.5 ml of hydrazine hydrate and stored for 18 hours at 50°C and for 5 hours at room temperature. The dipeptide hydrazide which crystallised out was filtered off, washed with ethanol and ether and dried. Yield: 15.5 g; melting point 179°-180°C; $[\alpha]_D^{25}$ = +4.2° (c = 1 in dimethylformamide).

(e) Tos-S-Benzyl-L-cysteinyl-O-benzyl-L-tyrosyl-L-leucyl--L-leucyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-N^G--tosyl-L-arginyl-glycinamide.

A solution of 0.95 g of Tos-S-benzyl-L-cysteinyl-O--benzyl-L-tyrosine hydrazide in 15 ml of dimethylformamide was treated at -20°C with 4.5 ml of 2 N hydrogen chloride in tetrahydrofuran and 0.8 ml of isoamyl nitrite. The mixture was stirred for 40 minutes at -20°C and treated at this temperature, after neutralisation with 1.01 ml of N-methylmorpholine, with a solution of L-leucyl-L-leucyl-L-asparaginyl--S-benzyl-L-cysteinyl-L-prolyl-N^G-tosyl-L-arginyl-glycinamide [obtained by cleavage of the Z-protecting group from 1.83 g of Z-L-leucyl-L-leucyl-L-asparaginyl-S-benzyl-L-cysteinyl-L--prolyl-N^G-tosyl-L-arginyl-glycinamide in the manner described in part (a)] in 10 ml of dimethylformamide. The mixture was stirred for 1 hour at -20°C and stored for 15 hours at 4°C. The mixture was then filtered and the protected nonapeptide precipitated by the dropwise addition of the filtrate to

water and filtered off. The precipitate was digested with boiling ethanol and filtered off and the residue dried. Yield: 1.2 g; melting point 228°-230°C; $[\alpha]_D^{25} = -24.8°$ (c = 1 in dimethylformamide).

(f) [Leu^3, Leu^4]-Argininevasopressin diacetate.

400 mg of Tos-S-benzyl-L-cysteinyl-O-benzyl-L-tyrosyl--L-leucyl-L-leucyl-L-asparaginyl-S-benzyl-L-cysteinyl-L--prolyl-N^G-tosyl-L-arginyl-glycinamide were reduced with sodium in 500 ml of liquid ammonia. After removal of the ammonia, the residue was dissolved in 600 ml of 0.2% acetic acid and the solution adjusted to pH 7.3 with sodium hydroxide. Thereupon, 55 ml of a 0.01 M potassium ferricyanide solution were added, the pH value being held at 6.8-7.4 by the addition of sodium hydroxide. The mixture was stored for 15 hours at 4°C and passed through a column of Amberlite IR-45 (Cl^- form). The eluate was acidified with acetic acid and adsorbed on Amberlite CG-50 (H^+ form). After washing with 500 ml of 0.2% acetic acid, the mixture was eluted with a mixture of pyridine/glacial acetic acid/water (30:4:66) and the eluate was lyophilised twice with intermediate uptake of water. For further purification, the lyophilisate was dissolved in 3 ml of a 0.5 M ammonium acetate buffer (pH = 6.4) and chromatographed again on a column of Amberlite CG-50 (H^+ form). The eluate was lyophilised several times. Yield: 115 mg; $[\alpha]_D^{25} = -10.3°$ (c = 1 in 1 N acetic acid).

Paper electrophoresis:

Buffer of 2 ml of glacial acetic acid and 20 ml of

pyridine made up with water to 1 litre (pH = 6.0): $R_{f(arginine)}$ = 0.64 ± 0.05;

Buffer of 37 ml of formic acid and 25 ml of acetic acid made up with water to 1 litre (pH = 1.7) = $R_{f(arginine)}$ = 0.47 ± 0.05.

Example 2

(a) β-Benzylthiopropionyl-L-tyrosyl-L-leucyl-L-leucyl-L--asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-N^G-tosyl-L--arginyl-glycinamide.

A solution of 0.373 g of β-benzylthiopropionyl-L-tyrosine hydrazide [prepared according to M. Zaoral et al, Collection Czech. Chem. Commun. 32, 1250 (1967)] in 10 ml of dimethylformamide was treated at -20°C with 3 ml of 2 N hydrogen chloride in tetrahydrofuran and 0.4 ml of isoamyl nitrite. The mixture was stirred for 40 minutes at -20°C and treated at this temperature, after neutralisation with 0.675 ml of N-methylmorpholine, with a solution of L-leucyl-L-leucyl-L--asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-N^G-tosyl-L-arginyl--glycinamide [obtained by cleavage of the Z-protecting group from 1.15 g of Z-L-leucyl-L-leucyl-L-asparaginyl-S-benzyl-L--cysteinyl-L-prolyl-N^G-tosyl-L-arginyl-glycinamide in the manner described in part (a) of Example 1] in 10 ml of dimethylformamide. The mixture was stirred for 1 hour at -15°C and stored for 3 days at 4°C. The mixture was then filtered and the protected peptide was precipitated by the dropwise addition of the filtrate to a mixture of water/ethanol (2:1), filtered off, redissolved in dimethylformamide,

reprecipitated by the dropwise addition of this solution to ethyl acetate, filtered off and dried. Yield: 0.8 g; melting point 215°-217°C; $[\alpha]_D^{25} = -36.1°$ (c = 1 in dimethylformamide).

(b) Deamino^1-[Leu^3, Leu^4]-argininevasopressin diacetate.

350 mg of β-benzylthiopropionyl-L-tyrosyl-L-leucyl-L--leucyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-N^G-tosyl--L-arginyl-glycinamide were converted into the desired deamino^1-[Leu^3, Leu^4]-argininevasopressin diacetate in a manner analogous to that described in part (f) of Example 1. Yield: 129 mg; $[\alpha]_D^{25} = -91.1°$ (c = 1 in 95% acetic acid).

Paper electrophoresis:

Buffer of 2 ml of glacial acetic acid and 20 ml of pyridine made up with water to 1 litre (pH = 6.0): $R_{f(arginine)} = 0.42 \pm 0.05$;

Buffer of 37 ml of formic acid and 25 ml of acetic acid made up with water to 1 litre (pH = 1.7): $R_{f(arginine)} = 0.24 \pm 0.05$.

Example 3

(a) Z-Glycyl-S-benzyl-L-cysteinyl-L-tyrosyl-L-leucyl-L--leucyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-N^G-tosyl--L-arginyl-glycinamide.

A solution of 0.465 g of Z-glycyl-S-benzyl-L-cysteinyl--L-tyrosine hydrazide [prepared according to K. Jost et al,

Collection Czech. Chem. Commun. 26, 2496 (1961)] in 10 ml of dimethylformamide was treated at -20°C with 2.4 ml of 2 N hydrogen chloride in tetrahydrofuran and 0.3 ml of isoamyl nitrite. The mixture was stirred for 40 minutes at -20°C and treated at this temperature, after neutralisation with 0.54 ml of N-methylmorpholine, with a solution of L-leucyl--L-leucyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-N^G--tosyl-L-arginyl-glycinamide [obtained by cleavage of the Z-protecting group from 0.92 g of Z-L-leucyl-L-leucyl-L--asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-N^G-tosyl-L-arginyl--glycinamide in the manner described in part (a) of Example 1] in 8 ml of dimethylformamide. The mixture was stirred for 1 hour at -15°C and stored for 2 days at 4°C. The mixture was then filtered and the protected decapeptide precipitated by the dropwise addition of the filtrate to a mixture of water/ethanol (4:1) and filtered off. The precipitate was redissolved in dimethylformamide, reprecipitated by the dropwise addition of this solution to ethyl acetate, filtered off and dried. Yield: 0.65 g; melting point 226°-230°C; $[\alpha]_D^{25}$ = -37.0° (c = 1 in dimethylformamide).

(b) Gly-[Leu^3, Leu^4]-argininevasopressin diacetate.

350 mg of Z-glycyl-S-benzyl-L-cysteinyl-L-tyrosyl-L--leucyl-L-leucyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl--N^G-tosyl-L-arginyl-glycinamide were converted into the desired Gly-[Leu^3, Leu^4]-argininevasopressin diacetate in a manner analogous to that described in part (f) of Example 1. Yield: 76 mg; $[\alpha]_D^{25}$ = -70.2° (c = 1 in 95% acetic acid).

Paper electrophoresis:

Buffer of 2 ml of glacial acetic acid and 20 ml of pyridine made up with water to 1 litre (pH = 6.0): $R_{f(arginine)} = 0.74 \pm 0.05$;

Buffer of 37 ml of formic acid and 25 ml of acetic acid made up with water to 1 litre (pH = 1.7): $R_{f(arginine)} = 0.49 \pm 0.05$.

The following Examples illustrate pharmaceutical preparations containing a polypeptide provided by the present invention:

Example A

Sublingual tablets of the following compositions were manufactured in a manner known per se.

a)	[Leu^3, Leu^4]-Argininevasopressin diacetate	5.83 mg
	Lactose	66.17 mg
	Sugar powdered	20.00 mg
	Polyvinylpyrrolidone	7.00 mg
	Magnesium stearate	1.00 mg
		100.00 mg
b)	[Leu^3, Leu^4]-Argininevasopressin diacetate	11.66 mg
	Lactose	71.34 mg
	Mannitol	60.00 mg
	Hydroxypropylmethylcellulose	5.00 mg
	Magnesium stearate	2.00 mg
		150.00 mg

Example B

An injection solution of the following composition was manufactured in a manner known per se.

	Per ml
[Leu^3, Leu^4]-Argininevasopressin diacetate	0.12 mg
Sodium chloride	9.00 mg
Hydrochloric acid 0.1 N ad pH 3.5	q.s.
H_2O ad inject.	ad 1.0 ml

Example C

A lyophilisate of the following composition was manufactured in a manner known per se.

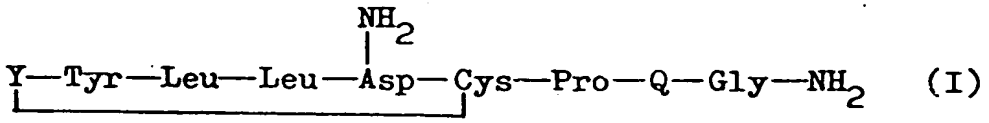
	Parts by weight
[Leu^3, Leu^4]-Argininevasopressin diacetate	11.60
L-Malic acid	1.74
D-Mannitol	150.00
	163.34

In order to obtain a ready-for-use injection solution, 163.34 mg of the lyophilisate are dissolved in 10 ml of distilled water.

~~Having now particularly described and ascertained the nature of our said invention and in what manner the same is to be performed, we declare that what we claim is:~~

The claims defining the invention are as follows:

1. A process for the manufacture of polypeptides of the general formula



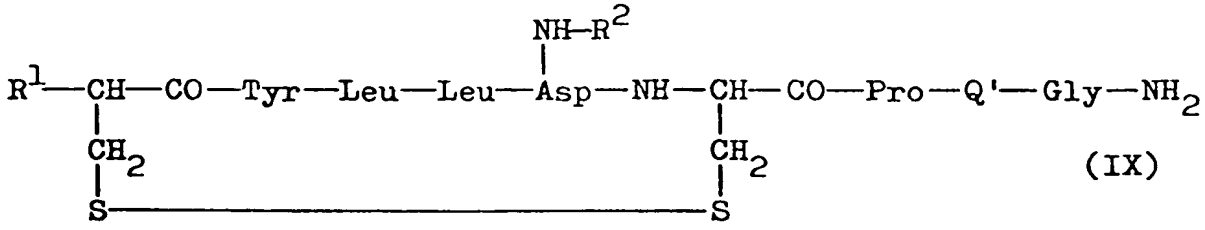
(I)

, wherein Q represents the residue of arginine or lysine and

Y represents the residue of cysteine, of β-mercaptopropionic acid or Gly—Cys— and wherein all amino acids with an asymmetric centre have the L--configuration,

and of pharmaceutically acceptable, non toxic acid addition salts thereof, which process comprises

a) cleaving off the protecting group(s) from a peptide of the general formula



(IX)

, wherein R^1 represents a hydrogen atom or a grouping of the formula R^{11}—NH—,

R^{11} represents a hydrogen atom, an

amino protecting group or an optionally protected glycyl residue,

R^2 represents a hydrogen atom or an amide protecting group,

Q' represents a grouping of the formula $-NH-CH[-(CH_2)_3-NH-C(=NH)-NHR^3]-CO-$ or $-NH-CH[-(CH_2)_4-NH-R^4]-CO-$,

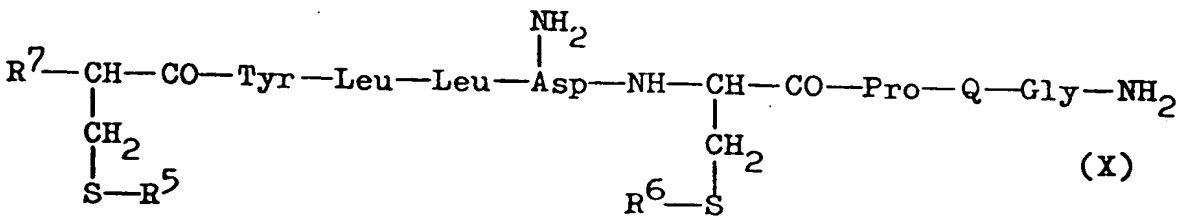
R^3 represents a hydrogen atom or a group protecting the guanidine residue and

R^4 represents a hydrogen atom or a group protecting the ε-amino group of lysine, provided that at least one of R^1, R^2 and R^3 or R^4 represents or contains a protecting group, and wherein all amino acids with an asymmetric centre have the L-configuration,

and, if desired, converting the free peptide obtained into a pharmaceutically acceptable, non-toxic acid addition salt by reaction with an organic or inorganic acid,

or

b) oxidising a peptide of the general formula



(X)

, wherein Q has the significance given earlier,

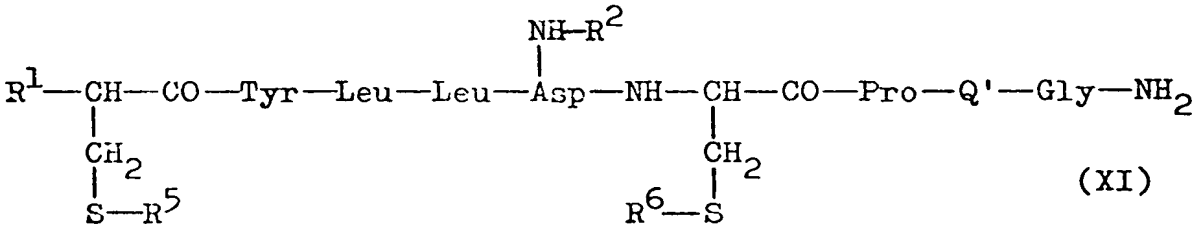
R^5 and R^6 each represent a hydrogen atom or a sulphydryl protecting group and

R^7 represents a hydrogen atom or the grouping H_2N- or Gly—NH— and wherein all amino acids with an asymmetric centre have the L-configuration,

with simultaneous or prior cleavage of protecting groups which may be present and, if desired, converting the product obtained into a pharmaceutically acceptable, non-toxic acid addition salt by reaction with an organic or inorganic acid,

or

c) oxidising a peptide of the general formula



(XI)

, wherein R^1 represents a hydrogen atom or a grouping of the formula R^{11}—NH—,

R^{11} represents a hydrogen atom, an amino protecting group or an optionally protected glycyl residue,

R^2 represents a hydrogen atom or an amide protecting group,

Q' represents a grouping of the formula

$-NH-CH[-(CH_2)_3-NH-C(=NH)-NHR^3]-CO-$ c.

$-NH-CH[-(CH_2)_4-NH-R^4]-CO-$,

R^3 represents a hydrogen atom or a group protecting the guanidine residue,

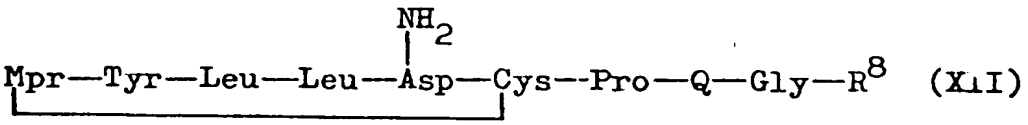
R^4 represents a hydrogen atom or a group protecting the ε-amino group of lysine and

R^5 and R^6 each represent a hydrogen atom or a sulphydryl protecting group, provided that at least one of R^1, R^2 and R^3 or R^4 represents or contains a protecting group, and wherein all amino acids with an asymmetric centre have the L-configuration,

with simultaneous cleavage of the protecting group(s) and, if desired, converting the product obtained into a pharmaceutically acceptable, non-toxic acid addition salt by reaction with an organic or inorganic acid,

or

d) amidating a compound of the general formula



(XLI)

, wherein Q has the significance given earlier,

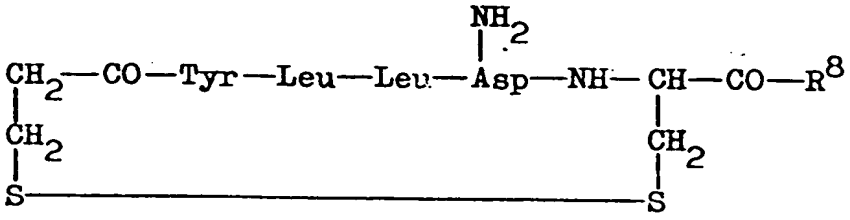
R^8 represents a hydroxy group or a

moiety activating the carboxyl group and

Mpr represents the residue of β-mercaptopropionic acid,

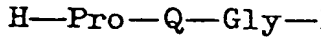
or

e) reacting a hexapeptide of the general formula



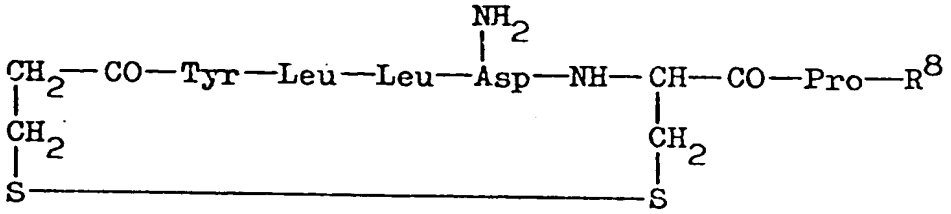
(XIII)

with a tripeptide of the general formula



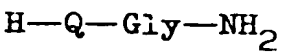
(XIV)

or reacting a heptapeptide of the general formula



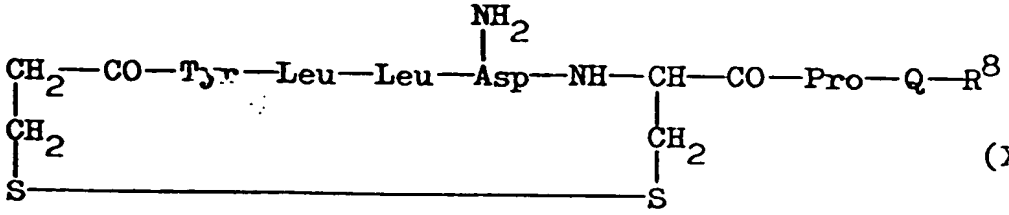
(XV)

with a dipeptide of the general formula



(XVI)

or reacting an octapeptide of the general formula



(XVII)

with glycinamide and, if desired, converting the resulting nonapeptide into a pharmaceutically acceptable, non-toxic acid addition salt, in formulae XIV and XVI Q having the significance given earlier and in formulae XIII, XV and XVII R^8 representing a hydroxy group or a moiety activating the carboxyl group and all amino acids with an asymmetric centre having the L-configuration.

2. A process according to Claim 1, wherein [Leu^3, Leu^4]-argininevasopressin or a salt thereof is manufactured.

3. A process according to Claim 1, wherein [Leu^3, Leu^4]-lysinevasopressin or a salt thereof is manufactured.

4. A process according to Claim 1, wherein deamino^1-[Leu^3, Leu^4]-argininevasopressin or a salt thereof is manufactured.

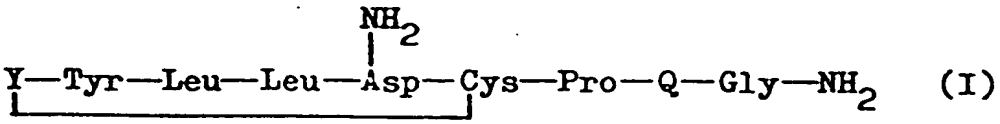
5. A process according to Claim 1, wherein deamino^1-[Leu^3, Leu^4]-lysinevasopressin or a salt thereof is manufactured.

6. A process according to Claim 1, wherein Gly-[Leu^3, Leu^4]-argininevasopressin or a salt thereof is manufactured.

7. A process according to Claim 1, wherein Gly-[Leu^3, Leu^4]-lysinevasopressin or a salt thereof is manufactured.

8. A process for the manufacture of the polypeptides set forth in Claim 1, substantially as hereinbefore particularly described, especially with reference to the foregoing Examples 1 to 3.

9. A process for the manufacture of pharmaceutical preparations, characterized in that one or more polypeptides of the general formula



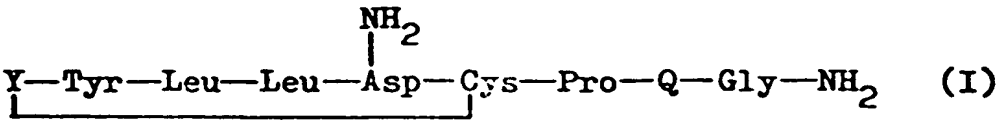
(I)

, wherein Q represents the residue of arginine or lysine and

Y represents the residue of cysteine, of β-mercaptopropionic acid or Gly—Cys— and wherein all amino acids with an asymmetric centre have the L--configuration,

or pharmaceutically acceptable, non-toxic acid addition salts thereof are mixed, as the active ingredients, with non-toxic, inert, therapeutically compatible solid or liquid carriers and/or excipients commonly used in such preparations.

9. Pharmaceutical preparations, characterized in that they contain one or more polypeptides of the general formula



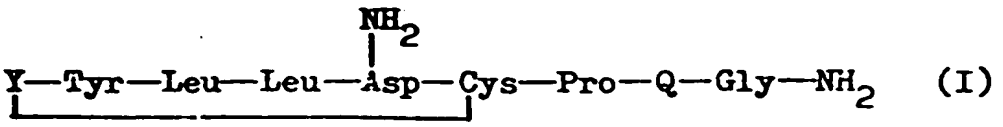
(I)

, wherein Q represents the residue of arginine or lysine and

Y represents the residue of cysteine, of β-mercaptopropionic acid or Gly—Cys— and wherein all amino acids with an asymmetric centre have the L--configuration,

or pharmaceutically acceptable, non-toxic acid addition salts thereof, and a pharmaceutically acceptable carrier.

11. A compound of the general formula



(I)

, wherein Q represents the residue of arginine or lysine and

Y represents the residue of cysteine, of β-mercaptopropionic acid or Gly—Cys— and wherein all amino acids with an asymmetric centre have the L- -configuration,

and pharmaceutically acceptable, non-toxic acid addition salts thereof, whenever prepared by the process as claimed in any one of Claims 1 to 8, or by an obvious chemical equivalent thereof.

12. [Leu^3, Leu^4]-argininevasopressin and pharmaceutically acceptable, non-toxic acid addition salts thereof, whenever prepared by the process as claimed in Claim 1, 2 or 8, or by an obvious chemical equivalent thereof.

13. [Leu^3, Leu^4]-lysinevasopressin and pharmaceutically acceptable, non-toxic acid addition salts thereof, whenever prepared by the process as claimed in Claim 1, 3 or 8, or by an obvious chemical equivalent thereof.

14. Deamino^1-[Leu^3, Leu^4]-argininevasopressin and

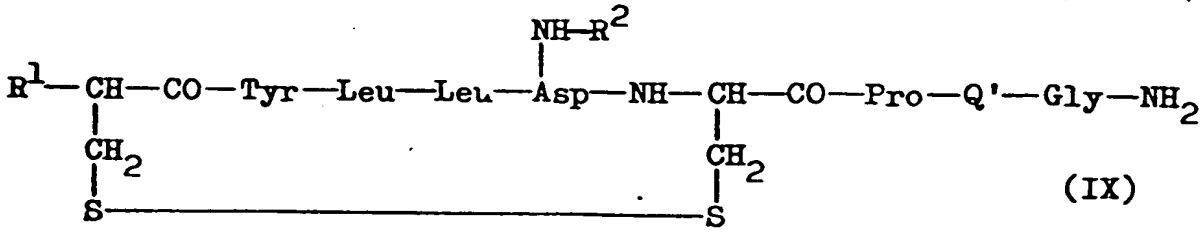
pharmaceutically acceptable, non-toxic acid addition salts thereof, whenever prepared by the process as claimed in Claim 1, 4 or 8, or by an obvious chemical equivalent thereof.

15. Deamino^{1}-[Leu^{3}, Leu^{4}]-lysinevasopressin and pharmaceutically acceptable, non-toxic acid addition salts thereof, whenever prepared by the process as claimed in Claim 1, 5 or 8, or by an obvious chemical equivalent thereof.

16. Gly-[Leu^{3}, Leu^{4}]-argininevasopressin and pharmaceutically acceptable, non-toxic acid addition salts thereof, whenever prepared by the process as claimed in Claim 1, 6 or 8, or by an obvious chemical equivalent thereof.

17. Gly-[Leu^{3}, Leu^{4}]-lysinevasopressin and pharmaceutically acceptable, non-toxic acid addition salts thereof, whenever prepared by the process as claimed in Claim 1, 7 or 8, or by an obvious chemical equivalent thereof.

18. A peptide of the general formula



(IX)

wherein R^1 represents a hydrogen atom or a grouping of the formula $R^{11}-NH-$,

R^{11} represents a hydrogen atom, an amino protecting group or an optionally protected glycyl residue,

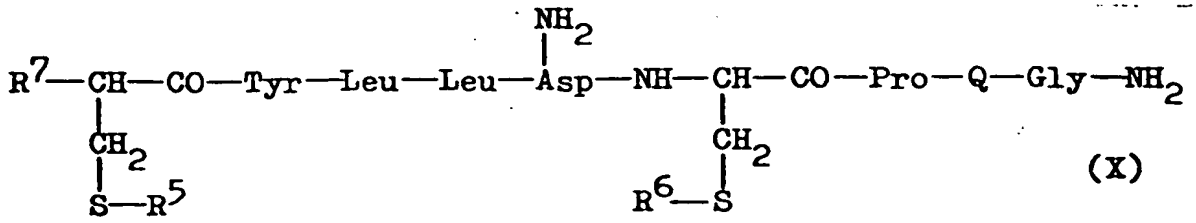
R^2 represents a hydrogen atom or an amide protecting group,

Q' represents a grouping of the formula $-NH-CH[-(CH_2)_3-NH-C(=NH)-NHR^3]-CO-$ or $-NH-CH[-(CH_2)_4-NH-R^4]-CO-$,

R^3 represents a hydrogen atom or a group protecting the guanidine residue and

R^4 represents a hydrogen atom or a group protecting the ε-amino group of lysine, provided that at least one of R^1, R^2 and R^3 or R^4 represents or contains a protecting group, and wherein all amino acids with an asymmetric centre have the L--configuration.

19. A peptide of the general formula



(X)

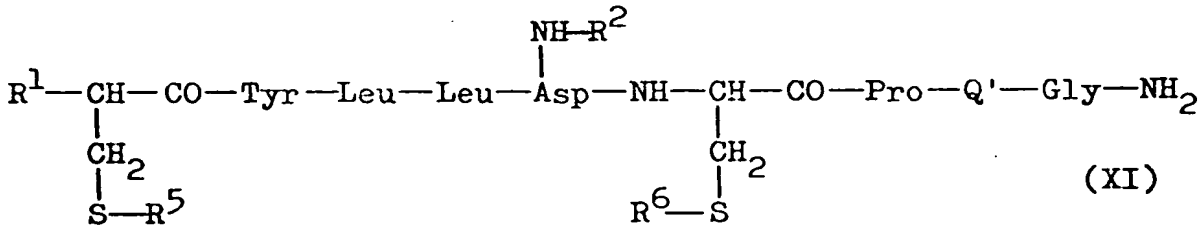
wherein Q represents the residue of arginine or lysine,

R^5 and R^6 each represent a hydrogen atom or a sulphydryl group and

R^7 represents a hydrogen atom or the grouping H_2N- or Gly—NH—, and

wherein all amino acids with an asymmetric centre have the L--configuration.

20. A peptide of the general formula



(XI)

wherein R^1 represents a hydrogen atom or a grouping of the formula R^{11}—NH—,

R^{11} represents a hydrogen atom, an amino protecting group or an optionally protected glycyl residue,

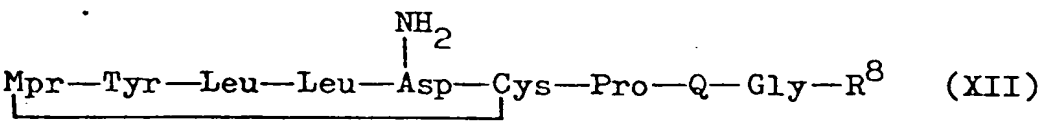
R^2 represents a hydrogen atom or an amide protecting group,

Q' represents a grouping of the formula $-NH-CH[-(CH_2)_3-NH-C(=NH)-NHR^3]-CO-$ or $-NH-CH[-(CH_2)_4-NH-R^4]-CO-$,

R^3 represents a hydrogen atom or a group protecting the guanidine residue and

R^4 represents a hydrogen atom or a group protecting the ε-amino group of lysine, provided that at least one of R^1, R^2 and R^3 or R^4 represents or contains a protecting group, and wherein all amino acids with an asymmetric centre have the L--configuration.

21. A compound of the general formula



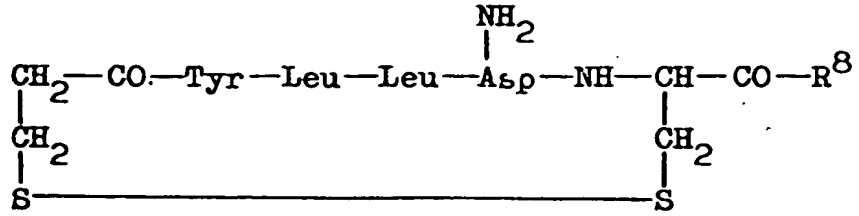
(XII)

wherein Q represents the residue of arginine or lysine,

R^7 represents a hydroxy group or a moiety activating the carboxyl group and

Mpr represents the residue of β-mercaptopropionic acid, and wherein all amino acids with an asymmetric centre have the L-configuration.

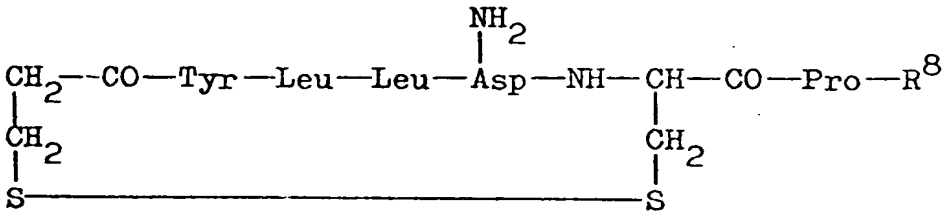
22. A hexapeptide of the general formula



(XIII)

wherein R^8 represents a hydroxy group or a moiety activating the carboxyl group and wherein all amino acids with an asymmetric centre have the L-configuration.

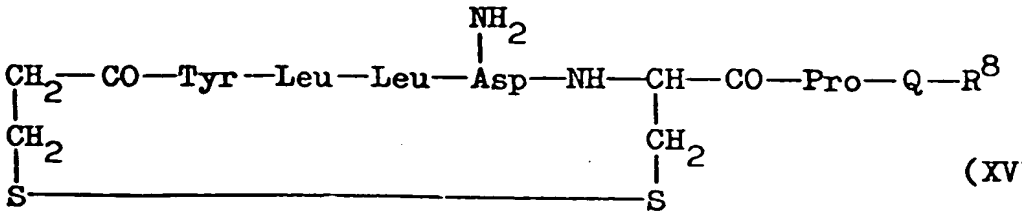
23. A heptapeptide of the general formula



(XV)

wherein R^8 represents a hydroxy group or a moiety activating the carboxyl group and wherein all amino acids with an asymmetric centre have the L-configuration.

24. An octapeptide of the general formula

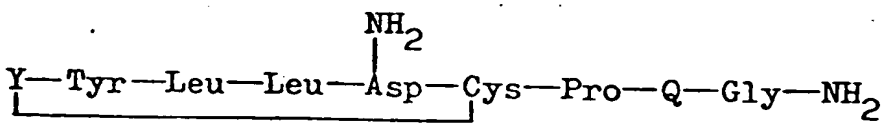


(XVII)

wherein Q represents the residue of arginine or lysine and

R^8 represents a hydroxy group or a moiety activating the carboxyl group and wherein all amino acids with an asymmetric centre have the L--configuration.

25. A compound of the general formula



(I)

, wherein Q represents the residue of arginine or lysine and

Y represents the residue of cysteine, of β-mercaptopropionic acid (Mpr) or Gly—Cys— and wherein all amino acids with an asymmetric centre have the L-configuration,

and pharmaceutically acceptable, non-toxic acid addition salts thereof.

26. [Leu^3, Leu^4]-argininevasopressin and pharmaceutically acceptable, non-toxic acid addition salts thereof.

27. [Leu^3, Leu^4]-lysinevasopressin and pharmaceutically acceptable, non-toxic acid addition salts thereof.

28. Deamino^1-[Leu^3, Leu^4]-argininevasopressin and pharmaceutically acceptable, non-toxic acid addition salts thereof.

29. Deamino^1-[Leu^3, Leu^4]-lysinevasopressin and pharmaceutically acceptable, non-toxic acid addition salts thereof.